applicable for selection as a position to alter in the acceptor variable region framework.

The relevant amino acid positions to change also can be selected, for example, based on proximity to a CDR. In certain contexts, such residues can participate in CDR conformation or antigen binding. Moreover, this criteria can similarly be used to prioritize relevant positions selected by other criteria described herein. Therefore, differentiating between residues proximal and distal to one or more CDRs is an efficient way to reduce the number of relevant positions to change using the methods of the invention.

Other criteria for selecting relevant amino acid framework positions to alter include, for example, residues that are known or predicted to reside in three-dimensional space near the antigen-CDR interface or predicted to modulate CDR activity. Similarly, framework residues that are known or predicted to contact opposite domain of the heavy (V_H) and light (V_L) chain variable region interface. Such framework positions can effect the conformation or affinity of a CDR by modulating the CDR binding pocket, antigen interaction or the V_H and V_L interaction. Therefore, selection of these amino acid positions as relevant for construction of the diverse population to screen can beneficially identify framework changes which replace residues having detrimental effects on CDR conformation or absorb detrimental effects of residues occurring elsewhere in the framework.

Finally, other framework residues that can be selected for alteration include amino acid positions that are inaccessible to solvent. Such residues are generally buried in the variable region and therefore capable of

influencing the conformation of the CDR or V_H and V_L interactions. Solvent accessibility can be predicted, for example, from the relative hydrophobicity of the environment created by the amino acid side chains of the polypeptide or by known three-dimensional structural data.

In addition to selecting the relevant framework positions, the method of conferring donor CDR binding affinity onto an antibody acceptor variable region framework also incorporates changes in the donor CDR amino acid positions. As with selecting the relevant framework positions to change, there is similarly a range of possible changes that can be made in the donor CDR positions. Some or all of the possible changes that can be selected for change can be introduced into the population of grafted donor CDRs to practice the methods of the invention.

One approach is to change all amino acid positions along a CDR by replacement at each position with, for example, all twenty naturally occurring amino acids. The replacement of each position can occur in the context of other donor CDR amino acid positions so that a significant portion of the CDR maintains the authentic donor CDR sequence, and therefore, the binding affinity of the donor CDR. For example, an acceptor variable region framework targeted for relevant amino acid positions changes as described previously, can be targeted for grafting with a population of CDRs containing single position replacements at each position within the CDRs. Similarly, an acceptor variable region framework can be targeted for grafting with a population of CDRs containing more than one position changed to incorporate all twenty amino acid residues, or a

fractional subset, at each set of positions within the CDRs. For example, all possible sets of changes corresponding to two, three or four or more amino acid positions within a CDR can be targeted for introduction into a population of CDRs for grafting into an acceptor variable region framework.

Single amino acid position changes are generated at each position without altering the remain amino acid positions within the CDR. A population of single position changes will contain at each position the varied amino acid residues, incorporated either randomly or with a biased frequency, while leaving the remaining positions as donor CDR residues. For the specific example of a ten residue CDR, the population will contain species having the first, second and third, continued through the tenth CDR residue, individually randomized or represented by a biased frequency of incorporated amino acid residues while the remaining non-varied positions represent the donor CDR amino acid residues. For the specific example described above, these non-varied positions would correspond to positions 2-10; 1,3-10; 1,2,4-10, continued through positions 1-9, respectively. Therefore, the resultant population will contain species that represent all single position changes.

Similarly, double, triple and quadruple amino acid position changes can be generated for each set of positions without altering the remain amino acid positions within the CDR. For example, a population of double position changes will contain at each set of two positions the varied amino acid residues while leaving the remaining positions as donor CDR residues. The sets will correspond to, for example, positions 1 and 2, 1 and 3, 1 and 4, through the set corresponding to the first